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THE STRENGTH AND COMPOSITION OF BLACKLEG VACCINES *

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A report of investigations carried out by this department on the strength and composition of some of the most widely known blackleg vaccines is here presented. One commercial vaccine consisting almost entirely of pathogenic spore-forming organisms, not *Bacillus chauvæi*, was subjected to a special study, since the strength of this vaccine differed markedly from that of all other vaccines.

The experiments were undertaken to determine, if possible, the fundamental reasons for so many unsatisfactory results from the use of blackleg vaccine. Complaints had been received concerning all vaccines in common use. Part of these involved losses of calves immediately after vaccination, others death from blackleg in spite of numerous vaccinations. The reports of nonprotection were especially numerous when the station followed the Kitt method of attenuating in streaming steam. When the vaccine was attenuated by dry heat, and hence was stronger than the steam-attenuated material, several notices were received of the vaccine's having apparently produced blackleg. From reports covering a period of 6 years the conclusion was drawn that there is no vaccine in use which does not justify serious complaints from one or both of these causes. After consideration of the opinions of various authorities we decided to measure the strength of the various blackleg vaccines on guinea-pigs.

Foth,¹ in speaking of his pure-culture vaccine (which would undoubtedly be far safer than any other form of vaccine because of the absence of contamination, which is known to increase the virulence of blackleg material, and also because of its fine trituration, making it even and regular in strength), states: "The spore-containing vaccine for direct subcutaneous injection should by no means be more than $\frac{1}{2}$ of the minimum lethal dose for guinea-pigs, when given subcutaneously in the ear. If the inoculation is performed in the neck instead of the ear, it seems advisable to reduce the dose to $\frac{1}{3}$ the minimum lethal dose for guinea-pigs. Due to the great expense inherent we have not been able to obtain data sufficient to make positive statements."

* Received for publication March 20, 1916.

¹ Ztschr. f. Hyg. u. Infektionskrankh., 1911, 10, p. 1.

Kitt² states that when the steam-attenuated vaccine was injected in doses of 30, 50, and 100 mg. it was not fatal for guinea-pigs, and the only reaction in cattle vaccinated subcutaneously with from 20 to 100 mg., was local edema, hardly noticeable.

In a personal communication Dr. J. R. Mohler of the U. S. Bureau of Animal Industry wrote in 1914 as follows: "The test used by the government bureau in the testing of their vaccine is that 3 guinea-pigs receive $\frac{3}{4}$ of the usual dose for calves, 3 guinea-pigs receive $\frac{1}{2}$ of the usual dose for calves, and 2 guinea-pigs receive $\frac{1}{3}$ of the usual dose for calves. If none die, the vaccine is good. A reaction is desired." In Dr. Mohler's opinion the variation in individual susceptibility of guinea-pigs plays a great part in preventing progress in establishing a standard.

In striking contrast with these views is the statement by King³ that "when results are correct those [guinea-pigs] which receive $\frac{3}{4}$ the usual dose, should die, those receiving $\frac{1}{2}$ should show symptoms and recover, while those injected with $\frac{1}{3}$ should remain normal."

TEST OF BLACKLEG VACCINES ON GUINEA-PIGS

Blackleg vaccines from a number of laboratories in this country and Europe were tested. The values given represent approximately the average fatal dose for a guinea-pig weighing from 9 to 13 ounces. The commercial vaccines are designated by letters, A, B, C, D, and E.

POWDER VACCINES

Bernese, powder, single.....	100 mg.	} fatal to guinea-pigs
Bernese, (1 guinea-pig)	40 mg.	
Lyonese, single	150 mg.	
B. A. I., Lot A (single).....	100 mg.	
B. A. I., Lot B (single).....	20 mg.	

Ten mg., after filtration, is the dose for yearlings. The Bernese vaccine was obtained by Dr. D. A. Guillebeau of Berne, Switzerland; the Lyonese was obtained through a Paris distributor; and the B. A. I. vaccine is the government vaccine, distributed by the U. S. Bureau of Animal Industry, Washington, D. C.

PELLET VACCINES

E vaccine.....	3 doses not fatal, 5 occasionally so.
D vaccine.....	3 doses not fatal; lethal dose not determined.
C vaccine.....	3 doses not fatal; lethal dose not determined.
A vaccine.....	3 doses not fatal, except of 1 serial, of which 1 or 2 doses were fatal. Severe losses occurred in several herds following the use of this serial of A vaccine.
B vaccine.....	1 dose regularly fatal.

The results of the tests carried out on the various pellet vaccines are here given in detail. It was difficult to ascertain just where the M.L.D. lay in many cases on account of the death of the guinea-pigs from

² Centralbl. f. Bakteriöl., 1888, 3, p. 572.

³ Quoted by Marshall, Microbiology, 1912, p. 472.

contaminating organisms when several pellets were given; this was not the case, however, with A single vaccine of which from 1 to 2 pellets were fatal, as this was practically free from contaminating organisms.

B Single Pellets.—Of a lot of 16 guinea-pigs receiving 1 pellet each of B. single vaccine, 2 lived and 14 died. B vaccine, single, was tested more thoroughly than any of the others. The tests included several different lots of vaccine.

B Double Vaccine Pellets.—Two pellets of the first of the double killed a guinea-pig. The second of the double was not fatal for a guinea-pig in small fractional doses of $\frac{1}{4}$ and $\frac{1}{8}$ pellet by weight, but was fatal in 1 of 2 guinea-pigs in a dose of $\frac{1}{2}$ pellet.

C Pellets.—A European single-pellet vaccine was found to be not fatal in guinea-pigs in doses of from 1 to 3 pellets, and occasional tests showed 5 to be safe. These pellets were of larger size and almost twice the weight of the B-pellet vaccine.

These vaccines, with the exception of B and C vaccine, were apparently made from blackleg-affected muscle.

E Single Pellets.—The guinea-pigs that received 1 pellet each, remained alive. Others that had received the same dose died from causes other than blackleg, no blackleg organisms being recovered.

A Single Pellets.—One pellet was not fatal for guinea-pigs.

These data show that the guinea-pig M.L.D. of the powder-form vaccines tested, varies from 20 to 150 mg., and that about 100 mg. are required of most of the vaccines to kill a guinea-pig.

These vaccine powders are mixed with water and filtered before use, 10 mg. of powder being extracted for each calf to be vaccinated. To what extent the filtration lessens the virulency has not been exactly determined. In some of these experiments the filtered vaccine was much weaker than the emulsion before filtration; in a few cases the filtrate was almost as virulent as the mixture before filtration. Even when making no allowance for weakening due to filtration, at least 2 doses of the strongest powder-form vaccine tested were required to kill a guinea-pig.

None of the pellets tested, with the exception of B, was fatal for guinea-pigs in doses of 3 pellets, the one serial of A vaccine, as already noted, being excepted. These observations on the use of A vaccine, as well as a series of experiments carried out at the station, have shown that serious vaccination losses may frequently follow the use of a blackleg vaccine on calves when the strength of the vaccine is such that one or two times the quantity administered to each calf is sufficient to kill a guinea-pig with blackleg. It will be noted that 1 pellet of B single vaccine was regularly fatal for guinea-pigs. In spite of this fact field observations indicate that very few vaccination accidents are attributa-

ble to B vaccine. This was at first considered remarkable, but on closer investigation it was shown that the virulence exhibited in guinea-pigs by the B vaccine was not due to *Bacillus chauvæi* but to another pathogenic spore-forming anaerobe, provisionally referred to as the bacillus of pseudoblackleg.

The presence of this pseudoblackleg bacillus in the B vaccine produces certain marked differences between this and the vaccine containing the blackleg bacillus, as shown here:

B VACCINE	VACCINES MADE FROM BLACKLEG MATERIAL
One dose of single regularly fatal to guinea-pigs.	One to 3 doses not fatal to guinea-pigs.
Sterile, i. e., no aerobic organisms.	Aerobic contamination; C pellets generally sterile.
Regular in strength.	Irregular in strength.
Does not rapidly lose strength with age.	Rapidly loses strength with age.
Safe in doses of several (5 to 7) pellets per calf.	Dangerous in doses of 1 pellet when of the same strength as B pellets.
Very few losses reported immediately following field vaccinations.	Many losses following field vaccination.
Reports covering its use in the field show poor protection.	Reports received at this station covering B. A. I. vaccine show fairly good field protection.
No blackleg organisms recovered from pellets or animals killed with same, tho numerous attempts were made.	Blackleg organisms recovered from nearly all pellets tested.
Pellets produce death in guinea-pigs that are thoroughly immune to blackleg.	After immunization to one strain of blackleg the guinea-pigs have been immune to all other strains as far as tested.
Foth's blackleg serum does not protect against this pill or against the pseudoblackleg cultures.	Foth's blackleg serum protects against all strains of blackleg tested.

These important findings in regard to B vaccine prompted a brief examination of the other vaccines (Table 1).

TABLE 1
BACTERIOLOGIC TESTS OF BLACKLEG VACCINES

Vaccine	Aerobic Organisms	Blackleg Organisms	Anaerobic Organisms Besides Blackleg
B single pellet { 1st pellet double vaccine 2nd pellet	Absent	Absent	Pseudoblackleg None
E single pellet.....	Present	Present	
C single pellet.....	Present in some cases	Present (?)	
D single pellet.....	Present in all cases..	Present	
A single pellet.....	Present	Present	
Foth's pure-culture vaccine..	Absent	Present in all cases..	
Bureau An. Ind., powder....	Present	Present in all cases..	
Lyonesse powder, single.....	Highly contaminated..	Present	
Bernese powder, single.....	Highly contaminated..		

B VACCINE AND THE ISOLATION AND DIFFERENTIATION OF THE PSEUDO-
BLACKLEG BACILLUS

To isolate the pseudoblackleg bacillus guinea-pigs were inoculated with 1 pellet of the B single vaccine. They succumbed within from 48 to 72 hours with a disease somewhat resembling blackleg. The essential differences between pseudoblackleg lesions and those of true blackleg were a heavy gelatinous exudate, and a slightly lighter discoloration of the affected parts in pseudoblackleg; in true blackleg the muscles were blacker, more spongy, and drier. The odor of blackleg was absent. The greenish cast over the affected areas, which resembled an advanced putrefaction, was typical of pseudoblackleg at the time of death. It was never noticed in pure blackleg, except from 12 to 18 hours after death, and then only in very warm weather.

The pseudoblackleg bacillus was the only one that could be cultured both from the pellets and from the animals inoculated with the pellets. Its morphology resembled that of the blackleg bacillus somewhat. It was a slightly thicker rod and had less tendency toward the early formation of spores and clostridia. The shape of the spores was slightly more oval, and larger staining areas surrounded the spores in their earlier formation than in blackleg. However, these differences were so nearly approached by nontypical organisms of each species that their diagnostic value is not absolute. The colonies in liver agar were irregular in outline, the blackleg colonies regular. The organism grew more luxuriantly than the blackleg organism, and under less strict anaerobic conditions. The gas formula in 4% glucose broth was $2\text{CO}_2/\text{H}$ to $3\text{CO}_2/\text{H}$; the gas formula for the pure blackleg cultures always showed more H than CO_2 . Pseudoblackleg blackened ferrous-sulfate agar, gave off a putrefactive odor, and was pathogenic in rabbits.

THE PATHOGENICITY OF PSEUDOBLACKLEG IN RABBITS

Ten rabbits of approximately the same weight and condition were selected and 5 of them inoculated intramuscularly with a culture of pure blackleg, the other 5 receiving, in the same manner, an equal dose of a pure culture of the pseudoblackleg organism. The cultures had been grown in the same media and under similar conditions. The pseudoblackleg culture had been isolated from a guinea-pig which had died from a B pellet. The culture of pure blackleg was of such virulence that 0.1 c.c. was uniformly fatal for guinea-pigs.

Three rabbits received 0.5 c.c. each of pure blackleg culture, No. 278, one rabbit received 1 c.c. and one received 1.5 c.c. None of the rabbits died or became even noticeably ill, while of the 5 rabbits receiving the same dose of pseudoblackleg only one remained alive. Pseudoblackleg organisms were recovered from the affected muscles of the rabbits that died. The B vaccine pellets were fatal for rabbits in 2 of 3 instances, when 2 pellets were given intramuscu-

larly in the thigh. Two rabbits similarly injected with 1 pellet each, remained well.

That the rabbits were as susceptible to pseudoblackleg as the guinea-pigs is evident if we consider the difference in weight between the two species. The lesions resembled those in the guinea-pigs, and might easily have been mistaken for the typical blackleg found in calves. The blackleg cultures and the pseudoblackleg cultures used in this experiment both killed guinea-pigs in doses of from 0.03 to 0.1 c.c., so that the different results with rabbits were not due to a difference in virulence between the cultures, but to fundamental differences in their pathogenic properties.

Inasmuch as the pseudoblackleg organism differed from the pure blackleg organism in cultural and pathogenic properties, experiments were undertaken to ascertain whether it possessed any power to immunize against *Bacillus chauvæi*. Numerous attempts were made by means of pure cultures, by extracts, and by fractional parts of pellets to render guinea-pigs immune to pseudoblackleg. (The records of experiments concerning the attempted immunization of calves against this vaccine will be published shortly.)

In most instances the guinea-pigs died after the 2nd or 3rd injection, and only a few were immunized against several fatal doses of pseudoblackleg. These guinea-pigs, immune to pseudoblackleg infection, readily succumbed to blackleg infection when tested with pure cultures of *Bacillus chauvæi*.

Since such difficulty was experienced in immunizing guinea-pigs against pseudoblackleg, another series of experiments was carried out in which guinea-pigs were immunized against various strains of pure blackleg and, subsequently, inoculated with pseudoblackleg cultures or B pellets.

The minimal lethal doses for guinea-pigs of the various cultures and vaccines used in this experiment were as follows:

Bernese vaccine	100 mg.
Lyonese vaccine	150 mg.
B. A. I. vaccine.....	100 mg.
Culture 219.....	from 0.02 to 0.1 c.c.
Culture 306	0.1 c.c.
Culture 298.....	from 0.05 to 0.1 c.c.
Culture 278.....	from 0.03 to 0.1 c.c.
Pseudoblackleg	0.1 c.c.

The lower figures were not always fatal, the M.L.D. varying from 0.02 to 0.1 c.c., which was fatal in over 95% of the cases. These figures are based on the results obtained in several hundred guinea-pigs. All strains of pure blackleg investigated had a guinea-pig M.L.D. constant within these limits when cultures were grown in a slightly modified form of von Hibler's brain medium.

Guinea-pigs were made highly immune to blackleg by means of blackleg vaccine and large doses of pure cultures; some received 4 doses at regular intervals during a period of 2 months, the last dose of pure blackleg culture representing 2.5 times the certainly fatal dose. Twenty-six days later they were inoculated with one uniformly fatal dose of pseudobackleg, and all promptly succumbed to infection by pure cultures of pseudobackleg isolated from B-vaccine pellets. Various blackleg vaccines, both domestic and foreign, including Foth's pure-culture vaccine and filtered blackleg muscle juice, were used to immunize the guinea-pigs so as to avoid the possibility of their not being immune to various strains of blackleg.

Guinea-pigs which had been similarly immunized against blackleg vaccine and cultures so that they would withstand several fatal doses of pure cultures of blackleg, were inoculated with 1 pellet each of B single vaccine, with fatal results showing that guinea-pigs highly immune to blackleg were not immune to the pseudobackleg cultures or pellets.

The failure of the blackleg-immune guinea-pigs to withstand 0.1 c.c. of the pseudobackleg cultures, or even a pellet of B single vaccine, indicates the absence of any considerable reciprocal immunizing action between the two organisms. These guinea-pigs had shown immunity to several different strains of *Bacillus chauvæi* before the test, so that their subsequent susceptibility to the organisms isolated from the pellets indicates that the organism which we have designated as pseudobackleg is, in reality, a separate species, and not a separate strain of a polyvalent organism. To what extent blackleg is polyvalent has not been determined, but no indications of a marked polyvalence have been encountered. Guinea-pigs immunized against Kansas strains of blackleg were immune against cultures isolated from vaccines obtained from Europe, and vice-versa.

The fundamental difference between the blackleg and the pseudobackleg is clearly indicated by experiments with blackleg immune serum, as given in the following. It was found that the blackleg immune serum protected against our pure culture No. 56, and that the same dose of the same serum failed to protect against the pseudobackleg culture. Many other strains of pure blackleg have been tested against this serum. In every instance the serum has protected the guinea-pigs whether the test dose has been pure culture, or the original blackleg meat. These strains were secured from widely different sources; some of them were isolated from blackleg vaccines obtained from Europe, others from blackleg vaccine manufactured at various places in the United States, and many from naturally occurring cases. The failure to find a single strain derived from such widely different

sources against which the serum would not protect seems to indicate that the polyvalence of the blackleg bacillus is of small importance.

The use of blackleg immune serum in differentiating *Bacillus chauvæi* from closely allied pathogenic anaerobes has been applied by Foth, Meyer,⁴ and others. It is considered by these investigators a very useful and reliable test. None of these investigators mentions any noteworthy degree of polyvalency of the blackleg strains with which they have worked.

Anti-infectious Blackleg Serum.—Anti-infectious blackleg serum made by the use of pure cultures of the blackleg organism and blackleg muscle juice was found to protect guinea-pigs in 1-c.c. doses against a naturally fatal dose of blackleg, while 1- and 2-c.c. doses of the same serum failed to protect against smaller doses of the pseudoblackleg cultures. The experiments were controlled in such a way as to prove the virulence of the cultures against which the serum was used.

SUMMARY

The powder-form vaccines must be given in amounts of from 20 to 150 mg. to kill a guinea-pig.

With 2 exceptions the commercial pellets were not fatal to guinea-pigs in doses of 3 pellets to a guinea-pig. One of these exceptions was an unusually strong serial of a vaccine the virulence of which had previously been low. This pellet produced severe vaccination losses. The other exception was that of B vaccine, which had a M.L.D. of 1 pellet, the strength of which was due to the presence of the pseudoblackleg bacillus.

The pseudoblackleg bacillus differs from *Bacillus chauvæi* in its pathogenicity in rabbits, in the formation of a putrefactive odor, in the size and appearance of the colony in pure culture, in the gas formula, in the blackening of iron-sulfate agar, in the pathogenicity in guinea-pigs highly immune to blackleg, and in the failure of blackleg immune serum to protect guinea-pigs against a lethal dose of pseudoblackleg culture or a B pellet.

⁴ Jour. Infect. Dis., 1915, 17, p. 458.